

STUDIES ON SYMPATHOMIMETIC AMINES. II. THE BIOTRANS-
FORMATION AND PHYSIOLOGICAL DISPOSITION OF D-AM-
PHETAMINE, D-P-HYDROXYAMPHETAMINE AND D-METH-
AMPHETAMINE

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STUDIES ON SYMPATHOMIMETIC AMINES. II. THE BIOTRANSFORMATION AND PHYSIOLOGICAL DISPOSITION OF D-AMPHETAMINE, D-P-HYDROXYAMPHETAMINE AND D-METHAMPHETAMINE

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There is little information concerning the fate of d-amphetamine, d-p-hydroxy-amphetamine (Paredrine) and d-methamphetamine, sympathomimetic amines with related structures. Previous studies on amphetamine were concerned with its urinary excretion in various animal species (Richter, 1938; Jacobsen and Gad, 1940; Beyer and Skinner, 1940; Keller and Ellenbogen, 1952).

The present report describes the metabolic fate and physiological disposition of d-amphetamine, d-p-hydroxyamphetamine, and d-methamphetamine in a number of animal species. It will be shown that these compounds are metabolized by a number of biochemical processes, including hydroxylation, demethylation, deamination, and conjugation and that there are considerable species variations in the transformation of these drugs.

CHEMICAL METHODS. *Estimation of Amphetamine.* Amphetamine is isolated from biological material at an alkaline pH by extraction into benzene. The amphetamine in the benzene extract is estimated spectrophotometrically by a modification of the methyl orange reaction (Brodie, Udenfriend and Dill, 1947a).

Procedure for urine. Pipet 1 to 5 ml. of urine (containing 5 to 50 microgm. of amphetamine) into a 60 ml. glass-stoppered bottle containing 0.5 ml. 1 N NaOH and 20 ml. benzene¹. Shake for 10 minutes. Transfer 15 ml. of the benzene extract to another 60 ml. glass-stoppered bottle containing 5 ml. of 0.2 M borate buffer, pH 10². Shake for 5 minutes. Transfer 8 ml. of the organic phase to a 12 ml. glass-stoppered centrifuge tube containing 0.4 ml. isoamyl alcohol¹. Add 0.6 ml. methyl orange reagent³ and shake for about 6 minutes. Centrifuge the tube and transfer 6 ml. of the benzene phase to a cuvet containing 1 ml. of a solution of 2 per cent by volume of sulphuric acid in absolute ethanol. Work as rapidly as possible to minimize the adsorption of the methyl orange complex on the sides of the tube. Determine the optical density in a spectrophotometer at 540 mμ.

Reagent blanks, run through the procedure are used for the zero settings. Standards are prepared by running known amounts of amphetamine through the procedure. Standards should be run concurrently with the unknown samples. An optical density of about 0.140 is obtained when 10 microgm. of amphetamine are assayed by this procedure (Coleman spectrophotometer Model 6A using 18 mm. cuvettes).

¹ Solvents are purified by successive washings with 1 N NaOH, 1 N HCl, and two washings with water.

² The alkaline wash serves to extract small amounts of methyl orange reacting material normally present in urine.

³ A stock solution of methyl orange reagent is made by dissolving 500 mgm. of methyl orange in 100 ml. of warm water. The methyl orange solution is washed several times with equal volumes of chloroform. An aliquot of the resulting solution is diluted with an equal volume of saturated boric acid immediately before use.

Procedure for tissues. Organ tissues are prepared by homogenization in 0.1 *N* NH4Cl and proteins are precipitated with trichloroacetic acid as described in a previous paper (Axelrod, 1953a). Centrifuge and transfer 5 ml. of the supernatant solution to a 60 ml. glass-stoppered bottle containing 1 ml. 2 *N* NaOH and 20 ml. of benzene. Proceed as described for urine.

Procedure for plasma. Pipet 5 ml. of plasma into a 40 ml. glass-stoppered centrifuge tube containing 1 ml. of 1 *N* NaOH and 12 ml. benzene¹. Shake for 10 minutes. Centrifuge the tube and transfer 10 ml. of the benzene phase into a 15 ml. glass-stoppered centrifuge tube containing 0.3 ml. isoamyl alcohol¹. Treat the benzene extract with methyl orange reagent as described in the urine procedure. Transfer 8 ml. of the benzene phase to a 15 ml. glass-stoppered centrifuge tube containing 1.5 ml. of 1 *N* HCl and shake for 5 minutes. Remove the organic phase by aspiration. Transfer 0.2 ml. of the HCl solution to a microcuvet⁴, and determine the optical density at 510 mμ in a Beckman spectrophotometer adapted for microspectrophotometry. A reagent blank run through the procedure should not give an optical density of more than 0.030 when 1 *N* HCl is used for the zero setting. An optical density of about 0.210 is obtained when 2 microgm. of amphetamine are assayed by this procedure.

Amphetamine added to urine and tissue in amounts from 10 to 50 microgm. and to plasma from 1 to 5 microgm. are recovered with adequate precision (98 ± 5 per cent).

Estimation of p-Hydroxyamphetamine. *p*-Hydroxyamphetamine is isolated from biological material by extraction at pH 9–10 into diethyl ether. The extraction is augmented by saturating the biological material with sodium chloride. The compound is then returned to 0.1 *N* HCl, treated with 1 nitroso-2-naphthol reagent (Gerngross *et al.*, 1933) and assayed spectrophotometrically at 520 mμ.

Procedure. Pipet 5 ml. of plasma or urine into a 120 ml. glass-stoppered bottle containing 2 to 3 gm. of sodium chloride. Adjust the pH to 9 to 10 by the addition of solid Na2CO3. Add 60 ml. of ether¹ and shake for 10 minutes. Centrifuge the bottle and transfer a 50 ml. aliquot of the ether layer to another 120 ml. bottle containing 5 ml. 0.1 *N* HCl. Shake for 5 minutes and transfer 4 ml. of the acid layer to a cuvet. Add 0.5 ml. of 0.1 per cent 1-nitroso-2-naphthol (recrystallized) in ethanol and 1 ml. nitric acid reagent (50 ml. of 2.5 *N* HNO3 plus 1 ml. 2.5 per cent NaNO2). After 45 minutes read the optical density at 520 mμ in a spectrophotometer. A blank containing 0.1 *N* HCl, 1-nitroso-2-naphthol and the nitric acid reagent is used to set the spectrophotometer to read 100 per cent transmission.

The distribution of *p*-hydroxyamphetamine in an ether-salt saturated buffer system pH 9 is such that with volumes of 50 and 5 ml., respectively, about 90 per cent of the phenol is extracted in the organic phase. Standards are therefore prepared by handling known amounts of *p*-hydroxyamphetamine in the same manner as the unknown solutions. Standards are run with each set of determinations since there is a small daily variation in the distribution ratio. An optical density of about 0.140 is obtained when 20 microgm. of *p*-hydroxyamphetamine are run through the procedure (Coleman Model 6A photoelectric colorimeter using 18 mm. cuvetts). Hydroxyamphetamine added to biological material in amounts from 5 to 200 microgm. was recovered with adequate precision (98 ± 6 per cent).

Estimation of total p-Hydroxyamphetamine (free and conjugated) in urine. Add 1 ml. conc HCl to 5 ml. urine in a 15 ml. graduated tube. Cover the tube with a glass marble and heat in a boiling water bath for 1 hour. Cool and transfer 5 ml. of the urine to a 60 ml. glass-stoppered bottle containing 2 to 3 gm. of NaCl and 0.8 ml. of 10 *N* NaOH. Adjust the pH to 9–10 with solid Na2CO3, add 60 ml. of ether, and proceed as described for the estimation of *p*-hydroxyamphetamine.

The heating of blank urine results in the formation of a small amount of material (equivalent to 2 to 5 microgm. of apparent *p*-hydroxyamphetamine per ml. of urine) which is extracted into ether and reacts with 1-nitroso-2-naphthol. A correction for the urine blank may be made by treating an aliquot of urine collected 24 hours prior to the drug administration by the procedure described above.

⁴ Microcuvettes for Beckman spectrophotometer can be obtained from Pyrocell Mfg. Co., New York, N. Y.

Paper chromatography. Amphetamine and p-hydroxyamphetamine were chromatographed on paper by a method similar to that used by Wichstrom and Salversen (1952). The chromatograms were developed with butanol, acetic acid, and water (50:40:10) and sprayed with diazotized p-nitroaniline.

EXPERIMENTAL. *Identification of d-amphetamine and d-p-hydroxyamphetamine in the urine of dogs after the administration of d-amphetamine.* Two dogs received 5 mgm. per kgm. of d-amphetamine sulphate intraperitoneally and the urine was collected for the subsequent 48 hours. An aliquot of the pooled urine was made alkaline with NaOH and extracted with three volumes of benzene. The benzene extract was washed with one-fourth volume of pH 10 borate buffer, 0.2 M. The benzene extract was reduced to a small volume at room temperature under a stream of air. The material reacting with methyl orange was subjected to a 24 transfer counter-current distribution, using equal volumes of 0.1 M pH 8.5 borate buffer and benzene. Under these conditions d-amphetamine has a distribution ratio of about 1. After the counter-current distribution the material reacting with methyl orange was determined in each tube. The total optical density of the methyl orange reacting material was plotted against the serial number of the tube. The distribution curve was identical with the theoretical distribution curve calculated for d-amphetamine from its experimentally established ratio (fig. 1) and indicates that the substance in the urine which reacted with methyl orange was almost entirely d-amphetamine.

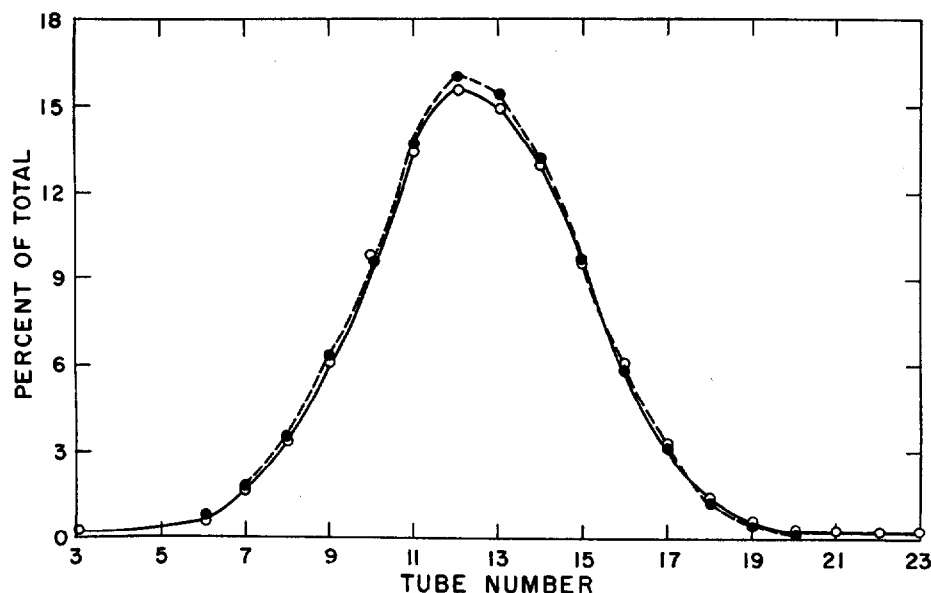


FIG. 1. Counter-current distribution of apparent d-amphetamine extracted from the urine of two dogs that received 5 mgm. per kgm. of d-amphetamine sulphate. Solid line represents the experimental distribution curve of apparent amphetamine extracted from the urine; dotted line the theoretical distribution curve for authentic d-amphetamine calculated from its experimentally established distribution ratio of 1.1. Solvents are benzene and 0.1 M borate buffer pH 8.5 (equal volumes).

The R_f (0.87) on paper chromatograms of the product extracted from urine was identical with that of authentic d-amphetamine.

The urine was examined for the presence of phenolic material in the following manner: An aliquot of urine was acidified with $\frac{1}{5}$ volume of HCl and heated in a boiling water bath for 1 hour. The urine was saturated with solid sodium chloride, adjusted to pH 9–10 with NaOH and solid Na_2CO_3 and extracted with 10 volumes of ether. The ether extract was reduced to a small volume under a stream of nitrogen and then shaken with 0.1 *N* HCl. The acid extract was adjusted to pH 8.6 with Na_2HPO_4 . The phenolic material was subjected to a 24 transfer counter-current distribution using equal volumes of pH 8.6 borate buffer 0.2 *M* and isoamyl alcohol. Under these conditions the material reacting with 1-nitroso-2-naphthol (see CHEMICAL METHODS) has a distribution ratio of about 1.7. After the counter-current distribution the amount of phenolic material was determined in each tube and plotted against the serial number of the tube (fig. 2). The distribution curve showed the presence of only one phenolic product having a distribution curve comparable to authentic p-hydroxyamphetamine. Further evidence for the identity of the apparent p-hydroxyamphetamine was obtained

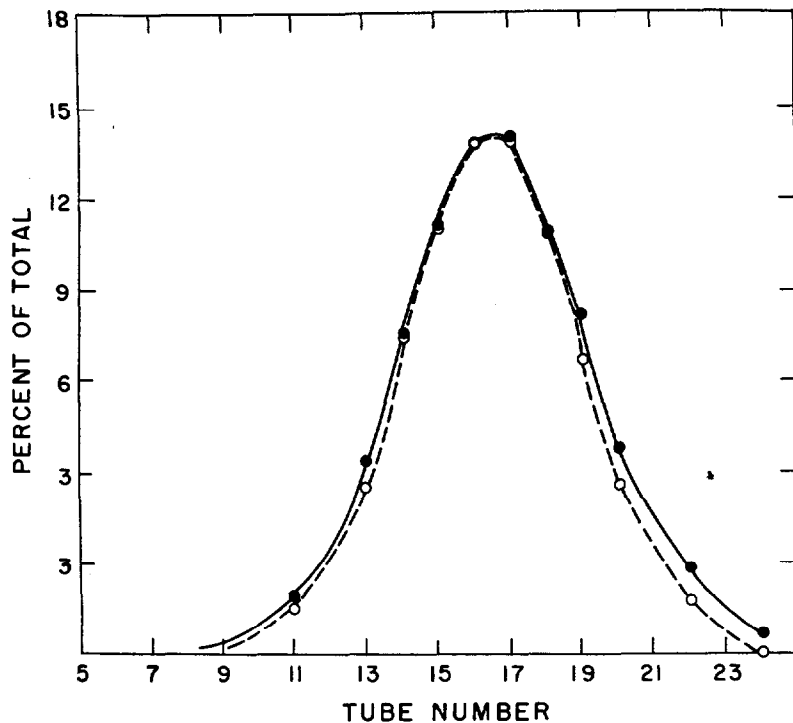


FIG. 2. Counter-current distribution of apparent d-p-hydroxyamphetamine isolated from the urine of dogs that received d-amphetamine. Solid line represents the experimental distribution curve of apparent d-p-hydroxyamphetamine extracted from the urine; dotted line the theoretical distribution curve for authentic d-p-hydroxyamphetamine calculated from its experimentally established distribution ratio of 1.7. Solvents are isoamyl alcohol and 0.2 *M* borate buffer pH 8.6. (equal volumes).

TABLE 1

The distribution of d-p-hydroxyamphetamine and apparent d-p-hydroxyamphetamine between pH 10 buffer and various solvents

The apparent p-hydroxyamphetamine was obtained from urine of dogs receiving d-amphetamine sulphate and d-methamphetamine hydrochloride respectively. The apparent p-hydroxyamphetamine was extracted from urine in the same manner as described under chemical methods. Aliquots of the aqueous HCl extract were adjusted to pH 10 and shaken with two volumes of organic solvent. The fraction of the compounds extracted is expressed as the ratio of amount of compound in the organic phase to total compound.

SOLVENT	AUTHENTIC D-P-HYDROXY-AMPHETAMINE	APPARENT D-P-HYDROXY-AMPHETAMINE AFTER D-AMPHETAMINE ADMINISTRATION	APPARENT D-P-HYDROXY-AMPHETAMINE AFTER D-METHAMPHETAMINE ADMINISTRATION
Isoamyl alcohol.....	0.88	0.89	0.88
Ether.....	0.34	0.35	0.32
Chloroform.....	0.13	0.13	0.14

TABLE 2

The metabolic fate of d-amphetamine in the dog

Recovery of d-amphetamine and its metabolic products from the urine of dogs given 5 mgm./kgm. of d-amphetamine sulphate, intraperitoneally.

The urine was collected over a period of 48 hours. The proportions of the various metabolites in the urine are expressed in percentage of the amount of d-amphetamine administered.

DOG	D-AMPHETAMINE	D-P-HYDROXY-AMPHETAMINE	CONJUGATED D-P-HYDROXY-AMPHETAMINE	TOTAL P-HYDROXY-AMPHETAMINE
	%	%	%	%
1	19	11	10	21
2	44	11	10	21
3	30	12	17	29
4	21	9	5	14
5	37	9	4	13

by comparing its distribution ratios between a number of organic solvents and pH 10 buffer with those of authentic p-hydroxyamphetamine (table 1). The results indicated that the two substances possessed the same solubility characteristics and were presumably the same compounds.

The apparent p-hydroxyamphetamine extracted into ether from the urine before and after acid hydrolysis had an R_f (0.68) on paper chromatograms identical with that of authentic p-hydroxyamphetamine.

The amounts of d-amphetamine and d-p-hydroxyamphetamine excreted in the urine of dogs after the administration of d-amphetamine. Five dogs received, intraperitoneally, 5 mgm. per kgm. of d-amphetamine sulfate. The urine was collected for the subsequent 48 hours and analyzed for d-amphetamine and its metabolic products (table 2). About 30 per cent of the drug was excreted unchanged; about 11 per cent as free p-hydroxyamphetamine and about 9 per cent as conjugated p-hydroxyamphetamine.

Plasma levels of d-amphetamine in dog. The plasma concentrations of d-amphet-

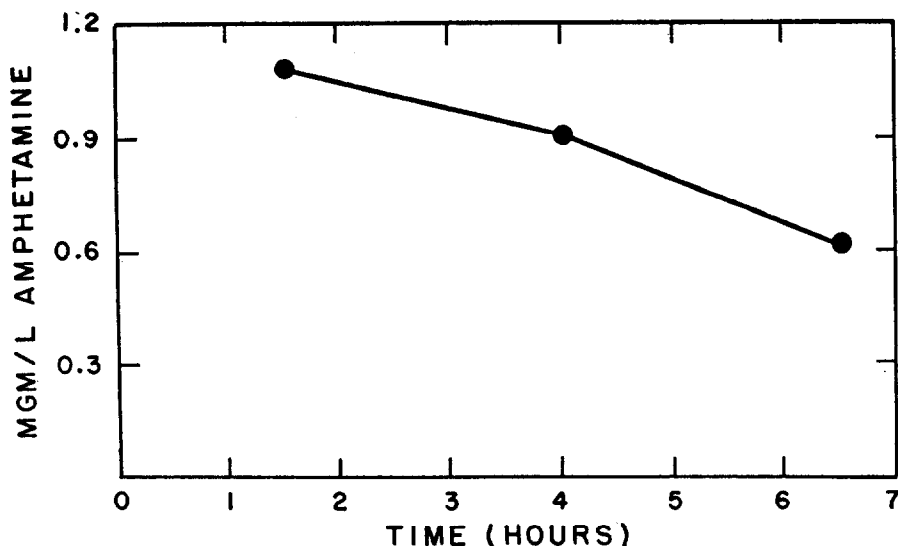


FIG. 3. Plasma levels of d-amphetamine after the intravenous administration of 5 mgm. per kgm. of d-amphetamine sulphate to a dog.

amine were measured at various time intervals following the intravenous administration of 5 mgm. per kgm. of d-amphetamine sulfate (figure 3, typical of four experiments). Plasma levels of amphetamine⁵ were low, and declined at a rate of about 8 per cent per hour. The low levels of amphetamine together with its slow rate of disappearance from plasma, suggested that the drug is extensively localized in organ tissues.

Tissue distribution of d-amphetamine. The distribution of d-amphetamine was examined in representative tissues of a dog which was given 10 mgm. per kgm. of the drug intraperitoneally. The animal was sacrificed by an intravenous injection of air, 60 minutes after the drug administration, and the tissues sampled immediately afterward (table 3). The drug was found to be localized in most organ tissues to a considerable extent. It was present in fat and bile in only negligible amounts. The concentrations of the drug in plasma and cerebrospinal fluid were the same indicating that there was no hindrance to the passage of the drug through the blood-brain barrier.

The extent to which d-amphetamine was bound to plasma proteins was determined by dialysis at 37° for 18 hours against isotonic phosphate buffer at pH 7.4. Visking membranes were used as dialysis bags. At plasma concentrations of 2 and 10 mgm. per liter approximately 15 per cent of the compound was found to be bound to the nondiffusible constituents of the plasma.

The fate of p-hydroxyamphetamine in the dog. The importance of p-hydroxyamphetamine (Paredrine) as a pressor agent and the role that the compound plays in the over-all metabolic transformation of amphetamine prompted a study of its fate in the body. Three dogs were given, intravenously, 10 mgm. per kgm.

⁵ d-Amphetamine, d-p-hydroxyamphetamine values in this report are expressed in terms of the free base.

TABLE 3

Distribution of d-amphetamine in dog tissues

Dog received 10 mgm./kgm. d-amphetamine sulphate intraperitoneally. The tissues were examined one hour after the administration of the drug.

TISSUE	D-AMPHETAMINE mgm./kgm.
Plasma.....	3.8
Cerebrospinal fluid.....	3.8
Liver.....	39.5
Lung.....	54.0
Spleen.....	35.5
Heart.....	14.5
Muscle.....	7.7
Brain.....	30.5
Kidney.....	68.0
Bile.....	3.5
Fat.....	1.6

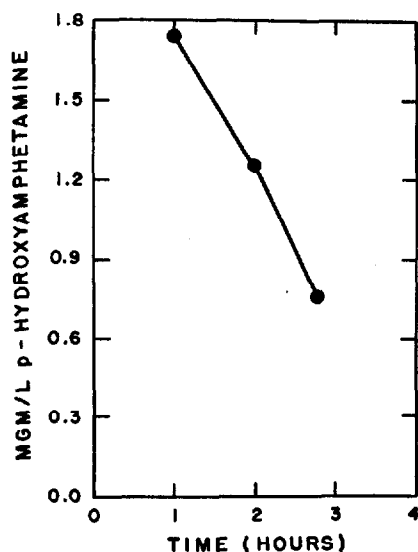


FIG. 4. Plasma levels of d-p-hydroxyamphetamine after the intravenous administration of 10 mgm. per kgm. of d-p-hydroxyamphetamine hydrobromide to a dog.

of d-p-hydroxyamphetamine hydrobromide and plasma levels and urinary excretion of the drug were measured. Plasma levels of d-p-hydroxyamphetamine⁵ declined rapidly at a rate of about 40 per cent per hour (figure 4, typical experiment) as compared with those of d-amphetamine which declined at a rate of about 8 per cent per hour (figure 3). About 30 per cent of the drug was found in the urine as free and 30 per cent as conjugated p-hydroxyamphetamine.

Fate of d-methamphetamine in the dog. The metabolic fate of d-methamphetamine was examined in two dogs which were given 5 mgm. per kgm. of the drug

intraperitoneally as the hydrochloride. Urine was collected for the subsequent 48 hours and examined for the presence of the parent drug and metabolic products. An aliquot of the pooled urine was made alkaline with NaOH and the basic material was extracted with three volumes of benzene. The benzene extract was reduced to a small volume at room temperature under a stream of nitrogen. The material reacting with methyl orange was subjected to a 50 transfer counter-current distribution using equal volumes of 1.0 *M* phosphate buffer, pH 8.0 and benzene. Under these conditions the methyl orange reacting material had a distribution ratio of about 1.0. After the counter-current distribution, the material reacting with methyl orange was determined in each tube. The total optical density of the methyl orange material was plotted against the serial number of the tube. The distribution curve indicated that at least two compounds reacting with methyl orange were present (figure 5). Tubes 6–12 contained material with partition coefficients similar to that of d-amphetamine and tubes 26–38 contained material with partition coefficients similar to that of d-methamphetamine. Further evidence for the identity of the apparent d-amphetamine in tubes 6–12 and d-methamphetamine in tubes 26–38 was obtained by the technique of comparative distribution ratios (Brodie *et al.*, 1947b). The distribution ratios of the methyl orange reacting compound in tubes 6–12 (d-amphetamine) and 26–38 (d-methamphetamine) between benzene and buffers at various pH values were the same as those of authentic samples (table 4). The R_f (0.86) on paper chromatograms of authentic d-amphetamine and the apparent d-amphetamine extracted from the urine with benzene were identical. From the above evidence it can be concluded that d-methamphetamine is demethylated to d-amphetamine in the dog.

The amounts of amphetamine and methamphetamine excreted in the urine

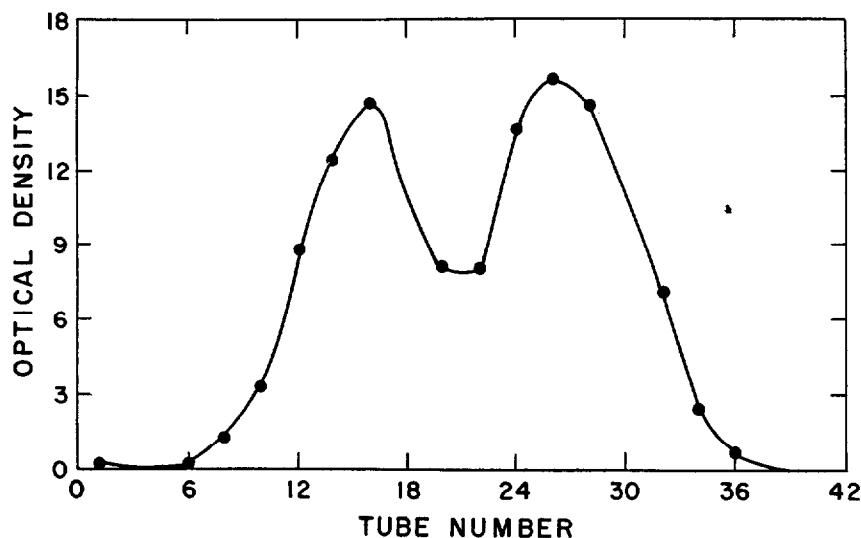


FIG. 5. Counter-current distribution of methyl orange reacting material extracted from the urine of dogs that received 5 mgm. per kgm. of d-methamphetamine hydrochloride. Solvents are benzene and 1.0 *M* phosphate buffer pH 8.0.

TABLE 4

The distribution of d-methamphetamine and d-amphetamine between benzene and water at various pH values

The apparent d-methamphetamine and d-amphetamine from the urine of a dog which received d-methylamphetamine was extracted and separated by benzene extraction and counter-current distribution. Aliquots of the benzene extracts in plates 6-12 (apparent d-amphetamine) and plates 26-34 (apparent d-methamphetamine) and authentic solutions of d-amphetamine and d-methamphetamine in benzene were shaken with half volumes of aqueous buffers at various pH values. The fraction of the compounds extracted at various pH values is expressed as the ratio of the amount of compound in the organic phase to total compound.

pH	AUTHENTIC D-AMPHETAMINE	APPARENT D-AMPHETAMINE	AUTHENTIC D-METHAMPHETAMINE	APPARENT D-METHAMPHETAMINE
8.6	0.61	0.60	0.84	0.82
8.0	0.33	0.34	0.62	0.60
7.4	0.15	0.15	0.27	0.27

after the administration of d-methamphetamine were calculated from the amount of methyl orange reacting material found in tube 16 (amphetamine) and tube 26 (methamphetamine), by application of a binomial expansion in the manner described by Williamson and Craig (1947). After the administration of d-methamphetamine about 18 per cent of the drug was excreted unchanged and about 15 per cent was demethylated to d-amphetamine. Correcting for the d-amphetamine which is further metabolized (table 2) it may be estimated that about 45 per cent of administered d-methamphetamine was demethylated to d-amphetamine in the dog.

The urine of dogs which received d-methamphetamine was also examined for the presence of phenolic material. The aliquot of the pooled urine was extracted with ether and subjected to a 24 transfer counter-current distribution in the same manner as described for the isolation of p-hydroxyamphetamine. The distribution curve indicated the presence of a single phenolic product having a partition coefficient and distribution curve similar to that of authentic d-p-hydroxyamphetamine. Further evidence for the identity of apparent d-p-hydroxyamphetamine was obtained by comparing its distribution ratios between a number of organic solvents and pH 10 borate buffer, with that of authentic d-p-hydroxyamphetamine (table 1). The apparent p-hydroxyamphetamine extracted into ether from the urine before and after hydrolysis had the same R_f (0.68) on paper chromatograms as that of authentic p-hydroxyamphetamine.

Analysis of the urine showed that about 10 per cent of the administered d-methamphetamine was excreted as free p-hydroxyamphetamine and 3 per cent as conjugated p-hydroxyamphetamine.

Fate of d-amphetamine and d-p-hydroxyamphetamine in a number of species. The metabolic fate of d-amphetamine was studied in guinea pigs, rats, and rabbits. Each animal received 5 mgm. per kgm. of d-amphetamine sulfate intraperitoneally and the urine was examined for amphetamine and p-hydroxyamphetamine (table 5). Dogs and rats excreted considerable amounts of both the unchanged amphetamine and its hydroxy derivative while guinea pigs and rabbits excreted

TABLE 5

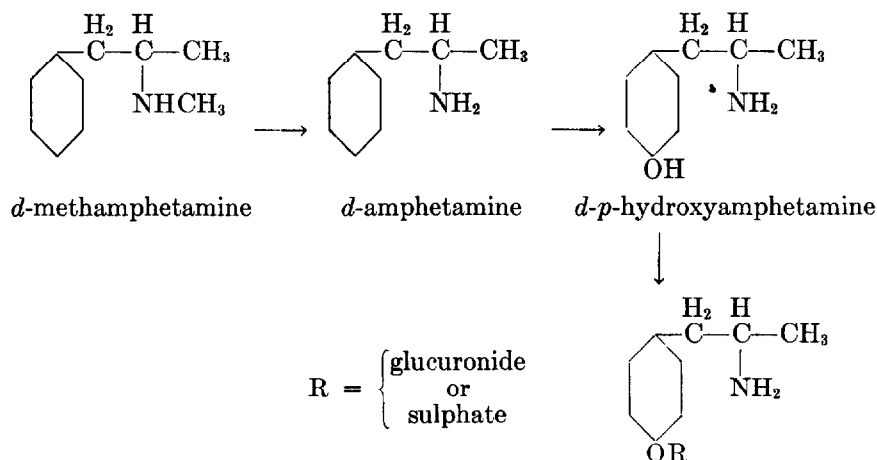
The fate of d-amphetamine and d-p-hydroxyamphetamine in a number of species

Five mgm./kgm. of d-amphetamine sulphate were administered intraperitoneally to each animal. The urine was collected over a period of 48 hours and examined for d-amphetamine and total p-hydroxyamphetamine (free and conjugated). Seven days later each animal was given 5 mgm. per kgm. of p-hydroxyamphetamine hydrobromide and the urine was examined for total p-hydroxyamphetamine (free and conjugated). The proportion of the various metabolites in the urine is expressed in percentage of the amount of the compound administered.

SPECIES	NUMBER OF ANIMALS USED	D-AMPHETAMINE ADMINISTERED		D-P-HYDROXYAMPHETAMINE ADMINISTERED	CALCULATED P-HYDROXYAMPHETAMINE FORMED AFTER D-AMPHETAMINE ADMINISTRATION
		d-amphetamine excreted	Total p-hydroxyamphetamine excreted	Total p-hydroxyamphetamine excreted	
		%	%	%	%
Dog.....	5	31	20	61	33
Rat.....	8	13	31	38	80
Guinea pig.....	4	0.7	0.5	69	0.7
Rabbit.....	4	1.2	3.6	55	6.5

only small amounts of unchanged drug and p-hydroxyamphetamine. To delineate further the intermediate metabolism of d-amphetamine the fate of d-p-hydroxyamphetamine was examined in dogs, guinea pigs, rats and rabbits. After the intraperitoneal administration of 5 mgm. of d-p-hydroxyamphetamine hydrobromide, dogs, guinea pigs, rabbits, and rats excreted 61, 69, 55 and 34 per cent of the drug, respectively, in both the free and conjugated forms, showing that the compound is relatively stable in these species. From these results it may be concluded that rat and dog hydroxylate amphetamine while guinea pigs and rabbits transform the compound through other metabolic pathways.

DISCUSSION. On the basis of the studies that have been described the following scheme for the metabolic transformation is suggested for d-methamphetamine, d-amphetamine, and d-p-hydroxyamphetamine.



The main route of metabolism of d-amphetamine in the dog involves hydroxylation to p-hydroxyamphetamine, a potent pressor agent. A considerable amount of administered d-amphetamine is excreted unchanged. Part of the p-hydroxyamphetamine is excreted in the conjugated form. d-Amphetamine disappears slowly in the dog as compared to its hydroxylated derivative, suggesting that in this species the major part of the pharmacological effect is due to the parent compound.

After the administration of methamphetamine to a dog, about 45 per cent of the compound is demethylated to d-amphetamine, part of which in turn is hydroxylated to p-hydroxyamphetamine. About 20 per cent of the administered methamphetamine is excreted unchanged. Previous studies on the metabolic fate of an analogue of methamphetamine, l-ephedrine, in the dog (Axelrod, 1953a) have shown that this compound is almost completely demethylated to norephedrine while small amounts of the drug are hydroxylated.

There are marked differences in the metabolism of d-amphetamine in different species. Dogs and rats hydroxylate considerable amounts of the drug. Rabbits and guinea pigs, on the other hand, apparently metabolize d-amphetamine by another pathway. Preliminary studies have revealed the presence of an enzyme in liver which can deaminate amphetamine to phenylacetone (Axelrod, 1954). Rabbit liver is considerably more active with respect to this enzyme than either rat, or dog, suggesting that amphetamine is extensively deaminated in the rabbit.

At present no studies on the metabolic fate of amphetamine have been made in man. Previous work has shown that a number of aromatic compounds, including acetanilide, aniline (Brodie and Axelrod, 1948) and antipyrine (Brodie and Axelrod, 1950) are hydroxylated in man; it seems likely that the same would hold true for amphetamine.

There are considerable differences in the mechanisms and rate of biotransformation of sympathomimetic amines in the dog. d-Amphetamine disappears slowly at a rate of about 8 per cent per hour by hydroxylation and renal excretion. l-Norephedrine is mainly excreted unchanged at a rate of 25 per cent per hour (Axelrod, 1953). d-p-Hydroxyamphetamine disappears at a rate of 40 per cent per hour by renal excretion and conjugation. l-Ephedrine is rapidly metabolized at a rate of 60 per cent per hour primarily by demethylation (Axelrod, 1953).

SUMMARY

Methods for the estimation of d-amphetamine and its metabolic product d-p-hydroxyamphetamine in biological materials are described.

In the dog and rat the main route of biotransformation of d-amphetamine involves hydroxylation of the aromatic nucleus to form p-hydroxyamphetamine, an active pressor agent. Hydroxylation of amphetamine occurs to a negligible degree in the guinea pig and rabbit.

d-Amphetamine disappears slowly in the dog compared to its hydroxylated derivative, suggesting that in this species the major part of the pharmacological effect of the drug is due to the parent compound.

A major route of metabolism of d-methamphetamine in the dog involves demethylation to d-amphetamine.

d-Amphetamine is bound to plasma proteins to a negligible degree but is highly localized in most organ tissues.

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